# Fine vascular anatomy of adult rabbit knee ligaments\*

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### INTRODUCTION

Since Hunter published the first description of the blood supply to articular structures in 1743, many investigations have focused on the vascular anatomy of diarthrodial joints. Some studies have described the general pattern of vessels in discrete articular ligaments (Alm & Stromberg, 1974; Arnoczky, Rubin & Marshall, 1979) tendons (Lundborg, Myrhage & Rydevik, 1977; Myrhage, Lundborg & Holmand Rydevik, 1979) and menisci (Arnoczky & Warren, 1982; Danzig, Resnick & Gonslaves, 1983) but the majority have been performed on synovial membranes (Davies & Edwards, 1948; Linstrom, 1963; Casley-Smith, Sims & Harris, 1976; Knight & Levick, 1982, 1983).

The microvasculature of articular connective tissue potentially supports a number of important physiological functions (Gardner, 1954; Liew & Carson-Dick, 1981). Vessels provide blood to tissue regardless of joint position or motion (Davies & Edwards, 1948). Vascular nourishment of normal and healing connective tissues occurs either directly (Lundborg, 1976; Arnoczky et al. 1979) or indirectly, through the formation of synovial fluid (Lundborg, 1976; Lundborg et al. 1977; Lundborg, Holm & Myrhage, 1980) and restoration of function in healing tissue is thought to depend largely on the vascular response to injury (Oegema, An, Weiland & Furcht, 1988). Synovium, for example, is richly supplied with blood vessels (Linstrom, 1963; Knight & Levick, 1983) and is the prime source of synovial fluid which nourishes and lubricates articular cartilage (McCutchen, 1978). Synovial microvascular beds are therefore clearly organised to function as diffusion networks (Lundborg et al. 1980; Knight & Levick, 1983). Less information is available on the microvascular anatomy of capsular and ligamentous tissue and beyond potential roles in cellular oxygenation and nourishment, much about its functional importance remains speculative.

Previous investigations have suggested that ligaments contain a limited blood supply, similar in some ways to synovial (Linstrom, 1963) and tendinous tissue (Alm & Stromberg, 1974; Myrhage et al. 1979; Carr & Norris, 1989), but evidence for this is fragmentary (Davies & Edwards, 1948; Liew & Carson-Dick, 1981). With the exception of the anterior cruciate ligament (ACL) (Alm & Stromberg, 1974; Arnoczky et al. 1979; Arnoczky, Tarvin & Marshall, 1982), little is known about the fine vascular anatomy of other discrete articular ligaments. Hence the purpose of this study is to describe in detail the microvascular anatomy of the medial collateral ligament (MCL),

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the lateral collateral ligament (LCL) and the ACL of the normal adult New Zealand White rabbit knee joint. Vascular perfusion with ink solution is used to visualise blood vessels in these structures. The objective of the study is to provide a qualitative description of microvascular anatomy so that studies using the rabbit knee joint as a model can be more clearly focused with respect to vascular changes following ligament injury. Some of these results have been published in abstract form (Bray et al. 1989).

#### MATERIALS AND METHODS

Six normal skeletally mature New Zealand White rabbits aged twelve months were studied (Riemans Ltd, Don Mills, Ontario). Rabbits were killed with an overdose of sodium pentobarbitone (Euthanyl, 2.5 ml/4.5 kg, M.T.C. Pharmaceuticals, Cambridge, Ont) and immediately after death the femoral artery and vein of each hindlimb were exposed approximately 6 cm above the knee joint. The femoral vein was transected completely and its proximal stump was tied off leaving the distal vein free to drain into an absorbent sponge. The femoral artery was cut transversely through approximately one-third of its circumference, tied off proximally, and cannulated with about 2 cm length of perfusion tubing (PE-60, I.D. 0.76 mm, O.D. 1.22 mm, Intramedic Corp). The tubing was secured in the vessel and to adjacent soft tissues by means of two circumferential sutures of 3.0 nylon.

With the knee held in 90° of flexion, the cannula and vessel were initially flushed with 20 ml of heparinised saline (10000 units/100 cc) at 37 °C, followed by injection of 20 ml of a mixture of 5% gelatin and ink (Higgin's Black Magic, A. W. Faber – Castell GMBH and Co, Stein/Nurnberg, West Germany) also at 37 °C. Flexion at approximately 90° was maintained by abutting the hind feet against a raised border on the bench counter with the animal supine. This prevented uncontrolled knee motion (as a result of muscle spasm during injections) and minimised the potential effects of changing ligament tension during perfusions. Perfusion was controlled by means of a Harvard pump (Harvard Apparatus, Model 901, Millis, Mass) at a nominal rate of 7.64 ml/min. Immediately following perfusion the entire hindlimb was disarticulated at the hip joint and stored at 4 °C for at least 4 hours prior to en bloc removal of the MCL, LCL and ACL. Ligaments were dissected sharply at their attachment sites so that bony insertions were excluded from the specimens.

Specimens consisted of the entire 'end to end' soft tissue substance of the ACL. MCL and LCL as well as a 2 to 5 mm border of adjacent 'epiligamentous' connective tissue. (Here we define the epiligament as any surrounding adherent connective tissue removed simultaneously with the ligament but which was grossly distinguishable from ligament tissue proper) (Fig. 1). Specimens were fixed with an excess (approximately 50 times tissue volume) of freshly prepared 10% phosphate buffered formalin (pH 7·4). After fixation (from two days to two months at 20 °C) specimens were frozen to -20 °C and serial sections were cut at 50  $\mu$ m in a parasagittal plane (Fig. 1) on a cryomicrotome (M.S.E, Pelcool 130035). Approximately 15 to 20 sections were obtained from each specimen. Sections were examined after being cleared in xylene and mounted for light microscopy. To strengthen the consistency of observations, all frozen sections were reviewed by three observers and consensus was obtained through inter-observer discussion. Vessels filled with ink were described with an orientation relative to the parasagittal plane of section. Vascular configurations could have been arterial, venous or capillary channels as no attempt to measure diameters was undertaken and perfusion did not distinguish between these vessel types.

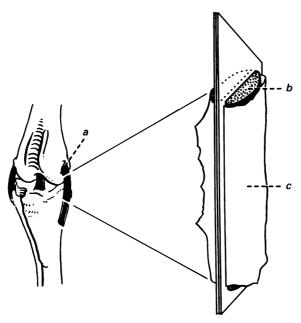


Fig. 1. Diagram of rabbit knee joint and sample specimen of the medial collateral ligament showing orientation used for cutting parasagittal sections. A similar orientation was used for anterior cruciate and lateral collateral ligaments. a, medial collateral ligament and epiligamentous tissue. b, ligament tissue proper. c, epiligamentous tissue covering ligament.

### **RESULTS**

Vascular injection of ink solution resulted in immediate filling of the skin and anterior investing fascia of the knee. When fascia overlying the knee joint was removed, medial and lateral collateral ligaments showed numerous filled vessels on the epilipamentous surface (Fig. 2A). The ACL showed a consistent but less abundant pattern of vessels arising in synovial tissue overlying the ligament (Fig. 2B).

During most perfusions, a brief phase of tonic flexion/extension movement occurred (muscle contraction in response to ink injection) potentially resulting in some alteration of ligament tension and also potentially expelling fluid from some filled ligamentous vessels.

Gross dissection of knees after ink injection demonstrated that the key sources of blood supply to the MCL and LCL were the superior and inferior geniculate arteries while, for the ACL, the middle geniculate artery was the primary source. Grossly visible vessels did not consistently cover the entire ligament surface in many specimens. Often, thin areas of epiligament appeared grossly devoid of filled vessels (Fig. 2A, B). On low power microscopic examination, however, a fine network of vascular plexuses could be identified in the epiligamentous tissue overlying most of the surface layer (Fig. 3).

Most vessels were found in the epiligamentous tissue surrounding ligament tissue proper. In collateral ligaments, epiligament tissue was distinguished by its gross location; on the joint side, epiligamentous tissue was largely formed of synovial membrane covering the ligament surface while epiligamentous tissue on the external ligament surface was largely formed of less dense connective tissue layers. In the ACL,





Fig. 2(A-B). Gross specimens following injection of ink solution. (A) shows a medial collateral ligament prior to removal. Note large vessels crossing the proximal part of the ligament and vascular plexuses in thick epiligamentous tissue overlying the insertion regions (arrows). These thicker epiligamentous regions contained the richest source of vessels surrounding the collateral ligaments (Fig. 3). (B) shows the injected rabbit knee after removal of the LCL, MCL, patellar tendon and menisci. Note the thin layer of ink injected synovium covering the ACL (arrow).

the epiligamentous layer was largely composed of synovial membrane and variable amounts of subsynovial connective tissue adjacent to the ligament but distinct as a separate surface layer on the ligament proper.

The epiligamentous plexus was formed outside the ligament tissue proper and gave rise to the majority of vessels observed to enter ligament tissue. In most ligament specimens, particularly the collaterals, the epiligamentous plexuses arborised into a branching anastomotic pattern, usually in the junctional region between superficial ligament and epiligament layers (Fig. 4). Different densities of these branching anastomotic vessels were observed along the length of ligament specimens. In general, areas of thick epiligamentous tissue (nearer to the bony insertion sites) exhibited the denser distributions of epiligamentous vessels (Figs. 2A, 3).

Epiligamentous vessels eventually gave rise to a smaller number of vascular channels some of which remained longitudinally orientated in the junctional region between the epiligament and superficial layers of the ligament while others appeared to penetrate more deeply into the ligament substance. Deeper vessels generally became longitudinally orientated with respect to the long axis of the ligament and formed a distinct, but sparse distribution (fewer, smaller vessels) deep within the ligament substance. This intraligamentous longitudinal plexus was most apparent in the LCL

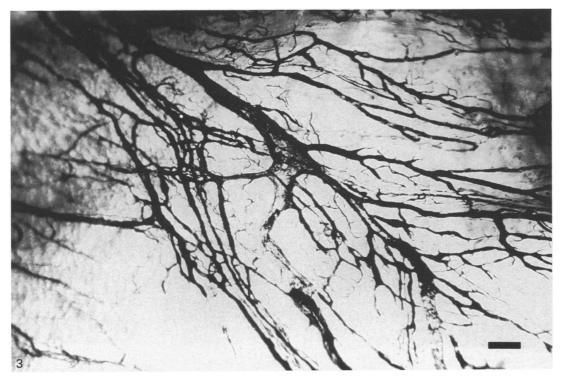


Fig. 3. Vascular plexus from injected epiligamentous tissue overlying a lateral collateral ligament. This tissue layer typically contained focal areas of branching anastomotic vessels. Bar, 50  $\mu$ m.

and MCL but the ACL demonstrated a similar, although less extensive, intraligamentous distribution of blood vessels. The ACL showed a very limited number of vessels, particularly in the entire central portion of the ligament midway between the proximal and distal bony insertion sites.

Serial sections of the ligament proper indicated a predominantly longitudinally orientated plexus of intraligamentous blood vessels. These vessels displayed a ladder-like anastomotic configuration and measured from 100 to in excess of 1000  $\mu$ m in length (Fig. 5). In sections showing linear anastomotic channels (Figs. 5, 6) the terminal regions often appeared to be 'blind ended'. However, on serial sectioning, these vessels were shown to pass out of the plane of section, continuing in a longitudinal direction within other layers of ligament tissue. Longitudinal ladder-like vessels were usually surrounded by avascular territories of ligament measuring up to 300 to 500  $\mu$ m between vessels. Many fields of ligament tissue examined were totally devoid of filled vessels. Since all ligament specimens were removed, excluding their bony attachment sites, no information was obtained on how these longitudinal vessels become distributed at ligament insertions.

Occasionally, within longitudinally orientated channels curious vascular anastomoses resembling glomus-like tufts, reminiscent of glomus tissue in skin (Bloom & Fawcett, 1975), were identified (Fig. 6). Similar vascular anastomoses were found in epiligamentous plexuses (Fig. 7). These glomus-like vascular anastomoses were observed in collateral as well as cruciate ligaments but were much less commonly seen than the linear channels described above (Fig. 5).



Fig. 4. Two areas of epiligamentous vessels feeding into a lateral collateral ligament. Large vessels arising from the epiligamentous plexus enter the ligament substance obliquely and then arborise into multiple (single arrow) vessels which eventually penetrate ligament tissue proper. The long axis of the ligament runs from left to right (double arrows). Bar,  $50 \mu m$ .

## **DISCUSSION**

This study used vascular perfusion with ink/gelatin solution to evaluate the fine vascular anatomy of specific knee ligaments in the adult rabbit. Sections were analysed from the ligament surface to deep within ligament substance. Although we examined the majority of ligaments from end to end, we did not include insertion sites and we therefore did not completely examine the vascularity in a longitudinal direction.

Limitations of vascular perfusion methods have previously been addressed (Linstrom, 1963; Liew & Carson-Dick, 1981). In addition to incomplete filling, overdistension with the possibility of incorrect interpretation may occur and one way vascular perfusions with ink do not distinguish (other than by gross diameters) between arterial, venous and capillary channels. We have not distinguished between these elements in this study. We recognise these limitations but nevertheless our results demonstrate a consistent pattern and distribution of perfused vessels in cruciate and collateral ligaments within and between animals. Serial section analysis of 50  $\mu$ m ligament sections has also added new information about how vessels enter and ramify deep within ligament tissue and has facilitated a more systematic, qualitative description of the fine vascular anatomy of individual knee ligaments.

We did not specifically examine whether some joint positions might be associated with increased or decreased vascularity, but this possibility has been addressed

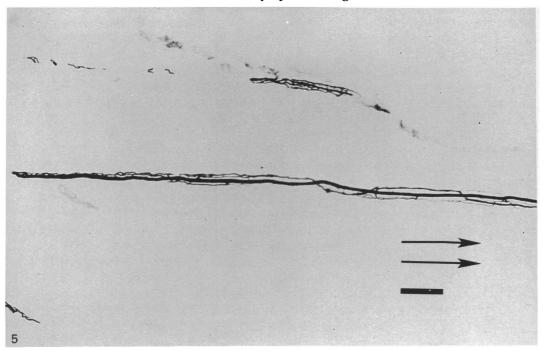


Fig. 5. Deep portion of a rabbit LCL showing two ladder-like anastomotic vessels running parallel to the ligament long axis (double arrows). Collagen bundles of extracellular matrix unstained and not visible. Note the large territories of ligament tissue apparently devoid of filled vessels. Bar, 50  $\mu$ m.

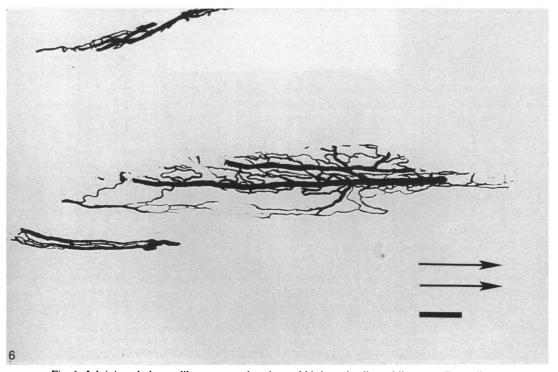


Fig. 6. Ink injected glomus-like structure deep in a rabbit lateral collateral ligament. Two adjacent ladder-like vessels are also seen. The long axis of the ligament is indicated by double arrows. Note that most of the surrounding ligamentous tissue appears devoid of filled vessels. Although some vessels appear truncated or blind ended, serial sectioning revealed them to be passing out of the plane of section. Bar,  $50 \ \mu m$ .

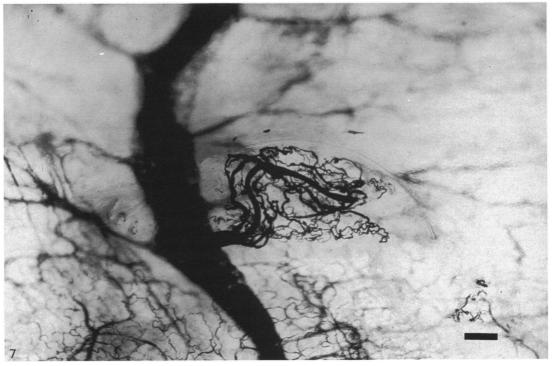


Fig. 7. Ink injected glomus-like structure arising from a large parent vessel in the epiligamentous tissue of a medial collateral ligament from a rabbit knee joint. Bar, 50  $\mu$ m.

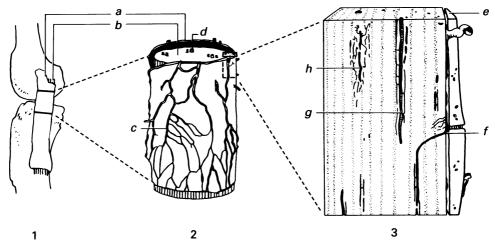


Fig. 8. Diagram of proposed fine vascular anatomy of adult rabbit medial collateral ligament (MCL). (1) Model of knee joint, medial view, showing MCL and epiligamentous tissue covering it. a, epiligamentous tissue layer. b, ligament proper. (2) Midsubstance region showing relationship between epiligament and ligament. c, vascular plexuses in epiligamentous tissue. Note the abundant blood supply and typical pattern of arborisation into small vascular channels. d, intraligamentous vessels. Deep ligament tissues typically have fewer vessels with a very different pattern and distribution from the epiligament. (3) Thin section model of MCL showing details of microvascular anatomy. e, epiligament at peripheral border of ligament proper. Note the relative abundance of vessels here compared to ligament substance. f, blood vessels entering ligament from epiligamentous plexus. g, typical linear anastomotic vessels deep in ligament substance. Vessels usually run parallel to ligament collagen bundles. h, glomus-like anastomosis found (infrequently) in ligament substance.

recently in a canine anterior cruciate ligament model where increasing ligament tension was associated with decreasing blood flow (Dunlap et al. 1989). It is therefore entirely possible that our results reflect vascular patterns which are specific to the experimental conditions and model described. This possibility warrants further investigation. We believe, however, that it will be necessary to approach such a problem with more quantitative methodologies than were used in this study to determine if, in fact, such a relationship exists in this model.

The vascular pattern in the epiligamentous and ligamentous tissues of the collateral and anterior cruciate ligaments demonstrated similarities in the way blood vessels enter discrete ligaments and subsequently become distributed into longitudinal channels. Anterior cruciate ligaments show proportionately a more scanty vascular supply, particularly in the central region (Fig. 2B), and this has been noted in other vascular studies of the ACL as well as in certain tendons (Arnoczky et al. 1979; Carr & Norris, 1989). However, nearer to the insertion sites, rabbit ACL clearly demonstrates some similarity to both the MCL and LCL, although the microvascular network of collateral ligaments is far more abundant and apparently more uniform throughout the length of the specimens. An 'idealized' pattern of entry and distribution of vessels is demonstrated schematically in Figure 8.

Epiligamentous tissues contain the most abundant vessels in the MCL, LCL and ACL of the rabbit. This tissue is variable in thickness but generally covers the entire surface of ligaments at the microscopic level. For the ACL, and for those surfaces of the MCL and LCL which are exposed to the joint cavity, the epiligament is essentially continuous with synovial membrane. The ACL would appear to be entirely enclosed by this synovial and subsynovial epiligament. The MCL and LCL, on the other hand, have surfaces outside the joint cavity which are similarly covered by epiligamentous fibrous connective tissue but are not associated with any synovial tissue. This external epiligamentous tissue lies adjacent to the more superficial gliding layers of fascia, aponeuroses and subcutaneous tissues covering the knee joint.

Epiligamentous vessels formed a net-like branching anastomotic pattern on the surface of all ligaments examined. An analogous layer found in tendon, the paratenon, also appears to contain the most abundant source of vessels, as shown in perfusion studies of this tissue (Scapinelli, 1968; Carr & Norris, 1989). These similarities further suggest that loose connective tissues adjacent to ligament and tendon contain a potentially important source of intraligamentous or intratendinous vessels. This speculation is supported by our results and by other investigations which suggest that a very limited number of epiligamentous vessels actually penetrate the ligament transversely to become true intraligamentous vessels (Scapinelli, 1968; Arnoczky et al. 1982).

Although ligament tissue has been described as relatively avascular (Davies & Edwards, 1948; Scapinelli, 1968) our results indicate a definite and consistent pattern of vessels deep within the substance of the rabbit MCL, LCL and ACL. Other reports have documented the presence of occasional large intraligamentous vessels (Davies & Edwards, 1948; Arnoczky et al. 1979) and similar observations have been made in tendon (Lundborg et al. 1977; Arnoczky et al. 1982; Carr & Norris, 1989), but the details of the fine vascular distribution within discrete rabbit ligaments has not been reported.

Intraligamentous vessels in the rabbit appear as longitudinal ladder-like anastomoses running parallel to collagen bundles in the tissue. Similar findings have been described in other models such as the canine anterior cruciate ligament (Arnoczky, 1983). While ladder-like vascular channels exhibit extensive longitudinal

distributions within the ligament substance, large areas of tissue appear to be devoid of filled vessels suggesting the possibility of relatively large, avascular territories within ligaments.

These avascular territories appear to be large, at least in rabbit ligaments. Since many sections were completely devoid of any filled vessels, the question must be raised whether the avascular regions are truly devoid of vessels or are simply unperfused territories. Given the consistency of avascular regions within and between animals and among different ligaments, we believe these avascular regions to be truly devoid of vessels. This is corroborated by other studies in rabbit ligaments where very few vessels have ever been described using routine haematoxylin and eosin-stained sections (Frank, Schachar & Dittrich, 1983). Further delineation of avascular territories and the possibility of variable perfusion of vessels, however, will require more quantitative assessments with careful control of variables which might influence regional perfusion in ligaments (joint position, ligament tension, perfusion pressure, etc). Similar observations of avascular territories have also been noted in tendon (Lundborg & Rank, 1978) and have led others to speculate on the importance of diffusional pathways for nutrition of normal and healing tissue (Lundborg et al. 1980; Whiteside & Sweeney, 1980). In addition to this presumed nutritive role we speculate that blood vessels have important physiological functions including maintenance of water content, extravasation of plasma components during inflammation and regulation of fluid and electrolyte balance in the extracellular matrix of these tissues.

The presence of multiple anastomotic channels in epiligamentous and ligamentous tissues raises more important questions about their physiological function. Gardner suggests that the abundance of nerves associated with these structures implies important neuroregulation of blood flow under various conditions such as growth and development, joint motion, inflammation and repair (Gardner, 1954). Linstrom, using *in vivo* vital staining techniques, observed 'glomerular tufts' and multiple anastomotic channels in rabbit synovium and considered these to be important in shunting circulation to different regions at different joint positions (Linstrom, 1963). Maintenance of tissue metabolic demands, regulation of local temperature and selective shunting of tissue microcirculation have all been suggested as possible functions of vascular anastomoses in joint tissues (Muratori, 1946; Davies & Edwards, 1948; Gardner, 1954; Linstrom, 1963; Liew & Carson-Dick, 1981). More detailed investigation of these anastomoses is necessary.

#### SUMMARY

The microvascular anatomy of discrete knee ligaments in adult rabbits is described. Epiligamentous plexuses give rise to a limited number of vessels which penetrate deeply into ligament substance. Intraligamentous vessels are usually longitudinally orientated, widely separated linear anastomoses but occasionally complex glomus-like configurations are present. The significance of these findings is discussed in relation to other articular connective tissues and to the possible roles of the intraligamentous microvasculature.

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